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(72) The inventors of this invention in the sense of being the actual devisers thereof within the meaning of Section 16 of the Patents Act 1949 are TAGE KJÆR NIELSEN of 101, Rørløkken, DK—2730 Herlev, Denmark, and ERIK KJÆR MARKUSSEN of 18, Tornekrogen, DK—3500 Værløse, Denmark, both Danish subjects.

(54) IMPROVEMENTS IN OR RELATING TO THE PRODUCTION OF ENZYME PREPARATIONS

(71) We, NOVO TERAPEUTISK LABORATORIUM A/S, a Danish company of 115, Nordre Fasanvej, 2200 Copenhagen N, Denmark, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for the production of enzyme preparations consisting of uniformly sized solid spheres.

In this specification and in the claims the expression "pellets" is intended to cover not only normal pellets, but also extruded, shaped bodies normally having an elongate structure, *e.g.* a spaghetti-like structure.

It is known to convert an extruded material into uniformly sized solid spheres by supplying the extruded pellets to a container with stationary solid side walls and a rotatably mounted bottom friction plate rotatable with a speed of from about 100 and up to 1800 rpm. This spheroidising is caused by centrifugal force and friction and has been performed in machines sold under the Trade Name MARUMERIZER obtained from the Eli Lilly Company and manufactured by Fuji Denki Kogyo Company, Osaka, Japan. The word MARUMERIZER is a Trade Mark.

We have now found that this spheroidising process is very useful in connection with enzyme preparations, particularly for use in the detergent industry, *e.g.* preparations comprising enzymes and additives normally used in washing and cleaning compositions, when the process is carried out with certain extruded enzyme-containing pellets. These pellets are produced in a conventional manner from a mixture of 75% to 97% of a solid enzyme-containing powder and 25% to 3% of water.

Accordingly, the process of the invention comprises subjecting enzyme-containing pellets prepared by extrusion from a mixture containing from 75 to 97 weight per cent of a solid enzyme-containing powder, comprising, if desired, an enzyme stabiliser, and from 25 to 3 weight per cent of water to a spheroidising process using a rotational speed of up to 2000 rpm in an apparatus causing centrifugal and frictional forces to be applied to the said pellets, whereafter, if desired, the solid spheres produced are subjected to a fluid-bed drying operation.

The enzyme preparations which can be produced by the process of this invention consist of particles of practically uniform size suitable for the intended industrial uses. The particles are substantially dust-free and show a sufficient mechanical strength for handling without the formation of dust. The particles also show sufficient flow properties for transportation in factories.

In the following examples rotational speeds of up to about 800—1000rpm are used during the spheroidisation, but speeds up to 2000 rpm may be employed.

In accordance with a preferred embodiment of the invention the spheroidising process is carried out in a machine of the type marketed under the Trade Name MARUMERIZER referred to above.

The product prepared by the process of the invention is easily soluble in hot as well as cold water. This is of special advantage when an enzyme product is to be used as an additive to a prewashing agent or a soaking agent.

The products of the present process possess a good storage stability, even under unfavourable conditions as regards temperature and humidity, and also when these products are used in perborate-containing washing agents.

If desired the products prepared in accordance with the invention may be further improved by coating in a manner known *per se* with a

[*Price 25p*]

tablet coating composition, e.g. as described in J. Am.Pharm. Association, Aug. 1954, Vol. XLIII, No. 8. Preferably the coating is carried out using a waxy substance, if desired a slightly sticky substance, but the coating agent should be easily soluble or dispersable in water.

Examples of preferred coating materials are as mentioned in the above literature polyethyleneglycol 6000 to 1000, but also nonylphenol-polyglycol-ethers having from 16 to 50 ethyleneglycol units, ethoxylated fatty alcohols in which the hydrocarbon moiety of the alcohol contains from 12 to 20 carbon atoms and the polyglycol moiety comprises from 15 to 80 polyethyleneglycol units, fatty alcohols, fatty acids and mono- and diesters of fatty acids and glycerol.

The optional coating process of the invention may be carried out in a simple and inexpensive apparatus, such as a mixing apparatus of the drum type having rotatable mixing aggregates. Thus, the use of complicated and expensive special kettles or fluidising units comprising nozzle arrangements can be avoided. Furthermore, it is often possible merely to melt the coating material and pour or spray it into the mixing drum, thus avoiding special solution processes.

The coated products are suitable for colouring with e.g. titanium dioxide or pigment colours, and the coated products are also properly protected against possible abrasion giving rise to the formation of undesirable enzyme-containing dust.

The enzyme-containing powder in addition to the enzyme itself preferably contains suitable additives, such as lubricants, fillers, binders and enzyme stabilisers. Polyethyleneglycols are examples of suitable lubricants, and examples of fillers are inorganic salts, for instance sodium chloride and sodium sulfate, pentasodium tripolyphosphate, tetrasodium pyrophosphate or the corresponding potassium salts, cellulose powder, starch powder, cellulose derivatives, starch decomposition products, starch derivatives, gelatine, casein, skimmed milk powder, polyvinyl alcohol and polyvinylpyrrolidones. Some of these substances may also act as binders. This applies for instance to the starch decomposition product dextrin, polyvinylpyrrolidone and polyvinyl alcohol. Gelatine, starch decomposition products, and other substrates for the enzymes and polyvinylpyrrolidone are examples of enzyme stabilizers. In particular, casein, skimmed milk powder and polyvinylpyrrolidone have been found to be useful.

Furthermore, polyvinylpyrrolidone acts in such a manner that each single string of extrudate becomes less adhesive so that the tendency to string adhesion in the spheroidising process is lowered.

In the spheroidising steps it can be advantageous to use a powdering agent so prevent any tendency of adherence between the spheroidising particles. Examples of such powdering agent are inorganic salts, such as anhydrous sodium sulfate, and inorganic oxides such as titanium dioxide.

The ratio between the enzyme powder and water in the mixture to be spheroidised depends on the enzymatic activity of the enzyme powder and the desired enzymatic activity of the final spheroidised enzyme product.

The following Examples illustrate the process of the invention. In some of these Examples we have used an enzyme concentrate called ALCALASE (the word "ALCALASE" is a Trade Mark), which is a commercial product and contains a proteolytic enzyme together with some inactive organic matter and some inorganic salts, mainly sodium sulphate. In an example, we have also used an enzyme concentrate called TERMOZYM (the word "TERMOZYM" is a Trade Mark), which is a commercial product and contains an amylolytic enzyme together with some inactive organic matter and some inorganic salts, mainly sodium sulphate.

Furthermore, the working Examples comprise examples showing the use of hemicellulose, fungal α-amylase as well as a proteolytic enzyme called ENZYME X and produced as described in our co-pending Cognate Application Nos. 45046/67 and 35921/68 (Serial No. 1,243,784) by cultivation of the *Bacillus* strain NCIB No. 10147 (NCIB No. 10147 is a deposit number for the said strain at the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland). Apart from the ENZYME X there may be used similar proteolytic enzymes prepared by aerobic cultivation of protease-forming species of the genus *Bacillus* on a nutrient medium having a pH within the range of 9 to 11 and maintaining during the main period of cultivation a pH in the said medium between 7.5 and 10.5, the said proteolytic enzymes showing a proteolytic activity of 80 to 100 per cent of maximum activity when measured at pH 12 by the Anso hemoglobin method carried out in the presence of urea. Furthermore, other amylases and proteinases, as well as milk-coagulating enzymes, cellulases, isomerases, e.g. glucose isomerase, pectinases, amylglucosidase and β-glucanase may be employed.

Example 14 is by way of comparison only.

The percentages in the Examples are per cent by weight.

Example 1.

There is produced a premix consisting of 30% of ALCALASE and 70% of sodium sulphate, and this mixture is moistened in a mixing aggregate with 8% of water which is sprayed on the mixture.

The moistened mixture is extruded in the conventional manner through a 0.7 mm screen.

and the pellets formed are then spheroidised in a MARUMERIZER at a starting speed of 400 rpm while powdering with 3% of titanium dioxide and finally at a speed of 800 rpm. Any traces of dust from the powdering substance can be removed by screening.

The final product has the following properties:

Proteolytic activity	1.3 Anson units/g
Particle size	0.7 mm
Bulk weight	about 1.0 g/cm³

The product is dust-free and soluble in aqueous media.

Example 2.

A premix consisting of 30% ALCALASE and 70% sodium chloride is moistened with 6% of water and extruded and spheroidised as described in Example 1. The final product has the same properties as those mentioned in connection with the final product produced in Example 1.

Example 3.

A premix having the following composition

25% ALCALASE
10% Dextrin
5% Cellulose powder
6% Polyethyleneglycol 6000
54% Anhydrous sodium sulfate

is moistened with 8% of water, and the moistened mixture is extruded in the conventional manner through a 0.8 mm screen. The pellets formed are spheroidised as in Example 1, except that anhydrous sodium sulphate is used as powdering agent instead of titanium dioxide.

The final product has the following properties:

Proteolytic activity	1.0 Anson units/g
Particle size	0.8 mm
Bulk weight	about 1.0 g/cm³

Example 4.

A premix consisting of 25% of ALCALASE, 10% of cellulose powder and 65% of sodium sulphate is moistened with 17.5% of an aqueous solution containing 10% of hydroxypropyl-cellulose and 2% of polyethyleneglycol 6000.

The moistened mixture is extruded and spheroidised as in Example 1, and the final product has the same properties as those mentioned in connection with the final product produced in Example 3.

Hydroxypropyl-cellulose may be substituted by polyvinyl-pyrrolidone.

Example 5

A mixture consisting of 33.5% of ALCALASE, 25% of TERMOZYM, 18% of dextrin, 18.5% of cellulose powder and 5% of polyethyleneglycol 6000 is moistened with 16% of water and extruded in the conventional manner through a 0.8 mm screen. The pellets formed are then spheroidised as described in Example 1.

The final product has the following properties:

Proteolytic activity	1.3 Anson units/g
Amylolytic activity	135 SKB units/g
Particle size	0.8 mm
Bulk weight	0.9 g/cm³

Example 6.

A powder mixture of the composition:

25% ALCALASE
10% Cellulose powder
3% Gelatine
60% Anhydrous sodium sulfate
2% Polyethyleneglycol 6000

is moistened with 16% of water and is extruded and spheroidised as described in Example 3. The final product has the same properties as those mentioned in connection with the product produced in Example 3.

Example 7.

A powder mixture of the composition

6.5% ALCALASE
10.9% Skimmed milk powder
82.6% Sodium chloride

was moistened with 18.5% of a solution of the composition

53% Water
35% Polyethylenegiycol 6000
12% Polyvinylpyrrolidone

The wetted mixture was extruded and spheroidised as described in Example 3.

The spheroidised product was fluid-bed dried at 40 to 50° C to a moisture content of about 0.5%.

The final product has the following properties:

Proteolytic activity	0.3 AU/g
Particle size	0.7 mm
Bulk density	1.05 g/cm³
Soluble in water	

Example 8.

Powder mixtures of the composition

5.5% ALCALASE
5.5% or 11% Casein
89% or 84.5% Sodium chloride

were moistened with 18.5% of a solution of the composition

53% Water
35% Polyethyleneglycol 6000
12% Polyvinylpyrrolidone

The wet mixture was extruded and treated as described in Example 3.

Example 9.

A premix of the composition

3.0% ENZYME X (prepared from strain NCIB No. 10147)
2.0% Polyvinylpyrrolidone
6.0% Polyethyleneglycol (6000)
89% Sodium chloride

was moistened with 8% of water and then extruded through a 0.9 mm screen and spheroidised at a speed of 1000 rpm.

The wet product was fluid-bed dried to a moisture content of approximately 0.5%.

The properties of the final product were

Proteolytic activity	1 KNPU/g
Particle size	appr. 0.8 mm
Bulk density	appr. 1.1 g/cm³
Water soluble	

KNPU represents Kilo NOVO-Proteinase Units. One NOVO-proteinase unit is defined as that amount of enzyme which, under standard conditions, hydrolyses casein at such a rate that the initial rate of formation of peptides giving a colour with TNBS (2,4,6-tri-nitrobenzene sulphonic acid-1-Na-reagent) corresponds to 1 umole glycine/minute. Standard conditions are: 0.5% Hammarsten casein (Merck) in 0.05 M borate buffer at pH 9.0 (measured at 20° C) reacting for 20 minutes at 50° C.

Example 10.

A premix of the composition

12% ALCALASE
5% Polyethyleneglycol 6000
1% Polyvinylpyrrolidone
84% Sodium citrate

was moistened with 9.3% of water and extruded, spheroidised and dried as described in Example 9.

The properties of the final product are

Proteolytic activity	0.5 AU/g
Particle size	0.7 mm
Water soluble	

Example 11.

A premix of the composition

2% Hemicellulose
6% Polyethyleneglycol 6000
2% Polyvinylpyrrolidone
90% Glucose

was moistened with 7% of water and extruded through a 0.9 mm screen and spheroidised at 900 rpm.

The wet product was fluid-bed dried at 40° C to a moisture content below 1%.

The properties of the final product were

Enzymatic activity	50,000 VHCU/g
Particle size	0.9 mm
Bulk density	0.8 g/cm³
Water soluble	

VHCU represents Viscosity Hemicellulose Units. The VHC method is a viscometric method, measuring the reduction of viscosity of a substrate made from locust bean gum under standard conditions. The viscosity is measured with an Ubbelohde viscometer.

Example 12.

A premix of the composition
35% Fungala-amylase
63% Sodium chloride
2% Polyvinylpyrrolidone

is moistened with 12% of water and extruded through a 0.8 mm screen and spheroidised at a speed of 800 rpm.

The spheroidised product was fluid-bed dried at 50° C, and the final product had the following properties

Enzymatic activity	1000 FAU/g
Particle size	0.7 mm
Bulk weight	about 0.9 g/cm³

Example 13.

A premix of the composition
26% ALCALASE
4% PLURONIC L 61
70% Sodium tripolyphosphate (MARCHON type d)

was moistened with 12.5% of water and extruded through a 0.9 mm screen. The words "PLURONIC" and "MARCHON" are Trade Marks.

The extrudate was spheroidised as described in Example 3.

The final product had the following properties

Proteolytic activity	1.0 AU/g
Particle size	0.9 mm
Bulk density	approx. 1 g/cm³
Soluble in water	

Example 14.

A premix of the composition

Bacterial amylase	15%
Polyethyleneglycol 6000	6%
Polyvinylpyrrolidone	2%
Sodium chloride	77%

was moistened with 5% of water and extruded through a 0.9 mm screen. The extrude was treated in the MARUMERIZER to form "muddles" each having a length of 1 to 3 mm.

Enzymatic activity 250 KNE/g
Particle size: Small cylinders having rounded-off end faces:
0.8 mm × 1—3 mm
Bulk density about 1 g/cm³

The granulate was dried in a fluid-bed at temperatures 40° C→60° C.

In the above Example, the treatment in the MARUMERIZER was carried out for about 2 minutes and this is the reason why the particles did not attain spherical form.

When the enzyme preparations prepared by the present process are intended for washing purposes, experiments made demonstrate that the storage stability in washing agents wherein enzyme preparation is present in the form of a finished, dried product is satisfactory, in particular when the enzyme stabilisers referred to in the foregoing are used.

The following residual activity tables including the references are in respect of finished, dried products prepared according to the invention, except only that the comparison Example represents powdered and not granulated ALCALASE not being spheroidised. The references are in accordance with the relevant Example except that the conventional substrate component for the enzyme has been omitted.

Storage stability in perborate-containing washing agent prepared on the basis of	Residual Activity on storing at 30°C; 70% rel. humidity for the periods shown. Method of analysis: TNBS			
	2 weeks	4 weeks	6 weeks	8 weeks
Example 7 (10.9% skimmed milk powder)	92%	74%	63%	62%
Reference to Example 7 6.5% ALCALASE, 2% PVP, 6% PEG 6000, Balance NaCl	84%	60%	52%	49%

	Residual Activity on storing 35°C; 67% rel. humidity for the periods shown. Method of analysis: TNBS			
	1 week	2 weeks	4 weeks	6 weeks
Example 7 (10.9% skimmed milk powder)	93%	78%	65%	—
Reference to Example 7	75%	65%	47%	—
Example 8 (11% Casein)	—	75%	64%	51%
Example 8 (5.5% Casein)	—	72%	54%	43%
Reference to Example 8	—	29%	24%	—
Comparison Example: ALCALASE, powdered ungranulared (double test)	—	30%	14%	13%

In the foregoing, the proteolytic activities have been determined by the Anson-method described in J.Gen.Physiol, *22*, 79—89 (1938). The TNBS-method for determining protease activity is described in J.Am.Oil Chem. Soc., 46: 81 (1969). α-amylase activities have been determined according to Cereal Chemistry *16*, 712 (1939), but with some modifications: thus, the following equations can be used for calculations:

1000 SKB units (pH 5.7)~53000 NOVO units for bacterial α-amylase and
1000 SKB units (pH 4.7)~37 FA units for fungal α-amylase

Hemicellulase activity has been determined viscosimetrically.

WHAT WE CLAIM IS:—

1. A process for the production of an enzyme preparation consisting of uniformly sized solid spheres, which process comprises subjecting enzyme-containing pellets prepared by extrusion from a mixture containing from 75 to 97 per cent by weight of a solid enzyme-containing powder comprising, if desired, an enzyme stabiliser, and from 25 to 3 per cent by weight of water to a spheroidising process using a rotational speed of up to 2000 rpm in an apparatus causing centrifugal and frictional forces to be applied to the said pellets.

2. A process according to Claim 1, wherein the solid spheres produced are subjected to a fluid-bed drying operation.

3. A process according to Claim 1 or 2, wherein the spheroidising process is carried out using a powdering agent preventing adhesion between the spheroidised particles.

4. A process according to Claim 3, wherein the powdering agent is an inorganic salt or an inorganic oxide.

5. A process according to any one of the preceding claims, wherein the spheroidising process is carried out in a machine having a cylindrical container with a stationary solid side wall and a rotatably mounted bottom friction plate rotatable at a speed up to 1800 r.p.m.

6. A process according to any one of the preceding claims, wherein the solid enzyme powder used comprises, as an enzyme stabiliser, (a) polyvinylpyrrolidone and (b) gelatine, casein, skimmed milk powder or other conventional substrate component for the enzyme(s).

7. A process according to any one of the preceding claims, wherein there is employed an enzyme powder wherein the enzyme is a protease, an amylase, an amyloglucosidase or and isomerase.

8. A process according to any one of Claims 1 to 6, in which there is employed an enzyme powder wherein the enzyme is a powdered bacterial proteinase prepared from

Bacillus licheniformis, a powdered amylase prepared from *Bacillus subtilus*, hemicelluiase, fungal α-amylase or a proteolytic enzyme prepared by aerobic cultivation of protease-forming species of the genus *Bacillus* on a nutrient medium having a pH within the range of 9 to 11 and maintaining during the main period of cultivation a pH in the said medium between 7.5 and 10.5, the said proteolytic enzymes showing a proteolytic activity of 80 to 100 per cent of maximum activity when measured at pH 12 by the Anson hemoglobin method carried out in the presence of urea.

9. A process according to any one of the preceding claims, wherein the enzyme-containing end product is coated with a tablet coating composition in a manner known *per se*.

10. A process according to Claim 9, wherein the end product is coated in a mixing apparatus of the drum type having rotatable mixing aggregates.

11. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 1.

12. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 2.

13. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 3.

14. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 4.

15. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 5.

16. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 6.

17. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 7.

18. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 8.

19. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 9.

20. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 10.

21. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 11.

22. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 12.

23. A process for the production of an enzyme preparation, in accordance with Claim 1 and substantially as described in foregoing Example 13.

24. An enzyme preparation consisting of uniformly sized solid spheres, which comprises enzyme-containing pellets prepared by spheroidisation of an extrudate of a mixture containing from 75 to 97 per cent of a solid enzyme-containing powder comprising, if desired, an enzyme stabiliser, and from 25 to 3 per cent of water.

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